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# Growth and Productivity Parameters of 4 Insertional-Mutant-rice Lines under Salinity Stress and their Insertion Patterns

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## Abstract

This experiment was aimed to observe the agronomy traits of 4 insertion-mutant-transgenic-Nipponbare rice lines in salinity stress condition and to evaluate the insertion patterns. The lines, (60, 170, 480 and 654), had been selected for their salinity tolerance from previous salinity test experiments. This experiment was conducted from the germination stage until the harvest time by planting the experiment plants in poly-bags and put them in flow tanks. The rice plants were treated with electrical conductivity of 3 mS/cm by means of NaCl using tap water. Nipponbare rice was used as wild type control. Amplifications for *hpt* and *bar* genes were conducted using their specific primers and the agronomy traits were measured of the experiment plants from germination stage, vegetative stage (plant height, tiller number) and reproductive stage (heading, seed number, productive tiller number). The observation of agronomic traits of the 4 mutant rice lines showed that each rice mutant lines of 60, 170, 480 and 654 has their own superior agronomic traits. However, the 170 rice mutant line is dominating the agronomic traits from germination and vegetative stage when treated with salinity stress. It has the smallest reduction percentages of germination, plant height in germination stage and length of flag leaves. In the productive stage, the line with the best reduction percentage for average of seed number per tiller was line 60; while the mutant rice line with the best reduction percentages of tiller number and plant height was line 654. The result of the PCR amplifications of *hpt* and *bar* genes showed different insertion patterns of each mutant line.

**Keywords:** Nipponbare, mutant, salinity, flowing tanks

## 1. Introduction

Abiotic stress is one of problems in utilizing marginal area for agricultural land [1]. One of the abiotic stresses is salinity level in the environment, which could affect the plant growth, resulting in yield loss. Salinity in the agricultural land can be caused by sea water intrusion, recirculating water treatment after pesticides and fertilizer application and excessive drought [2].

Rice, is sensitive to saline environment [3], although salinity tolerance varies from one growth stage to another stage [2, 4]. Germination is one of important stage in plant development. Any stress or disruption in this stage could affect the further development of the plant [5].

Ac/Ds transposon insertional mutagenesis in rice had been done in several experiments as reported by Greco et al. [6,7]. We have developed rice mutant lines population with Ac/Ds transposon insert for Nipponbare cultivar [8]. Screening on salinity tolerance; had been done for around 1000 rice mutant lines [9] resulting in a list of 10 rice mutant lines with best phenotype performances under salinity stress

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condition. In this experiment we used 4 lines with best performances out of those 10 mutant lines.

The aims of this experiment were to validate the salinity tolerance of 4 rice lines based on their agronomic traits under Ec 3 mS/cm from sowing until harvesting stage and to analyze the insertion patterns of those 4 rice mutant lines for further molecular analysis

## 2. Methods

**Material** The rice lines used in this experiment were 4 salinity-tolerant-candidate-mutant-rice line: 60, 170, 480, 654 and Nipponbare wild type. The seeds were germinated in 1:1 proportion of soil and manure in polybags. Three seeds were germinated in each of polybag. They were treated under flowing saline water prepared using sea salt purchased from local ornamental fish store,. There were 8 replications for each mutant lines and the nipponbare wild type both in saline and non-saline treatments as controls. The Ec of saline water (3mS/cm) was controlled by adding sea salt or tap water, accordingly. As control, one set of experiment under tap water only (without saline condition) was maintained at Ec: 800  $\mu$ S/cm

**Parameters** From germination stage to the harvest stage, data of vegetative and reproductive parameters were collected. In germination stage plant height and germination percentages were measured. During the vegetative stage total tillers and plant height were measured. As for reproductive stage flowering time and total grain productivity were measured. Reduction percentage of (some what parameters?) parameters were calculated using the following formula:

$$\text{Percentage of reduction} = \left( 1 - \left[ \frac{\text{data of treated plants}}{\text{data of control plants}} \right] \right) \times 100\%$$

**PCR for molecular marker analysis** 16 samples of genomic DNA (50 – 100ng) from each line was amplified using *hptII* and *bar* specific primer in the total of 12.5 $\mu$ l reaction; containing 1x DreamTaq PCR ready mix and 0.8  $\mu$ M primers (each of forward and reverse). The amplification of *hptII* gene were performed using primers HPT-F1 (5'-GCATCTCCCGCCGTGCAC-3'); and HPT-R1 (5'-GATGCCTCCGCTCGAAGTAGCG-3') under PCR condition as followed: 2 minutes of 95°C of initiation reaction, followed by 36 cycles of 95°C for 1 minute denaturing, 62°C for 1 minute annealing, 72°C for 1 minute extension; and terminated with 72°C for 7 minutes. Amplification of the *bar* gene were performed using primers BAR-48-F (5'-ACCATGAGCCCAGAACGACGC-3') and BAR-540-R (5'-CAGGCTGAAGTCCAGCTGCCAG-3') using PCR condition as above. The results were run in 0.8% agarose gel electrophoresis.

## 3. Result and Discussion

### 3.1. PCR and insertion patterns

To understand the pattern of insertion in the mutant rice plants, DNA were isolated and amplified the marker gene, *hptII* and *bar*. The *hptII* gene is linked to *Ac* element, while *bar* gene is linked to *Ds* element (Figure 1).

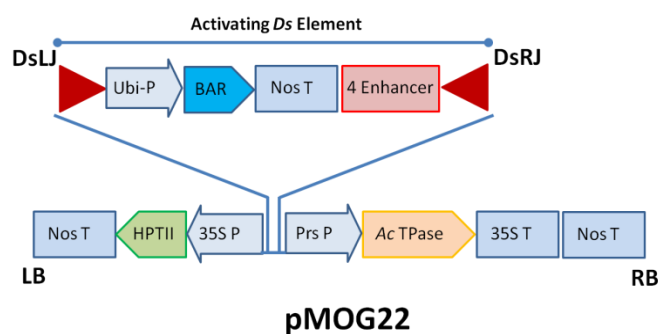


Fig 1. Map of pMOG22 with Ac/Ds transposon . This plasmid was inserted in rice cv nipponbare background to obtain nipponbare insertional mutant library. Plasmid were provided by Dr. Andy PereiraPRI Wageningen, the Netherlands

In this experiment all of the mutant lines are harbouring Ac element in their genomes. All of the mutant lines have the *hptII* gene. Among the mutant lines only 170 and 480 that have *Ds* element in their genome, indicated by the *bar* gene amplicons . From the electrophoregram (Figure 2), we could analyze the insertion patterns of each mutant line (Table 1).

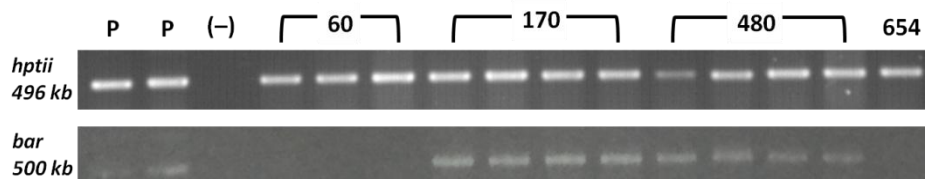


Fig 2. Electrophoregram of amplification of *hptII* gene (above) at 496 bp and amplification of *bar* gene (below) at 500 bp of pMOG22 (P), Nipponbare wild type as control negative plants (-), rice mutant lines 60, rice mutant lines 170, rice mutant lines 480 and rice mutant line 654.

Table 1. Insertion patterns of salinity-tolerant-candidate-insertional-mutant-rice lines.

Lines	<i>hptII</i>	<i>bar</i>
60	(+)	(-)
170	(+)	(+)
480	(+)	(+)
654	(+)	(-)

(+) = gene was amplified from the genome DNA of the mutant rice line,

(-) = gene could not be amplified from the genome DNA of the mutant rice line

This information of insertion pattern for each lines is important to understand the type of insertion, whether it is Ac, Ds or Ac and Ds. The Ac or Ds only insertion indicated that there were Ac transposon activities that excised Ds and may indicate the stability of the insertion. The Ac and Ds double insertion pattern could indicate that the insertion may not be stable yet, and therefore transposon activity may still be obtained. However, data from the PCR amplification results throughout the generation of T1-T4 concludes that even though there are still the presence of both Ac and Ds transposon in line 170 and 480, the transposon activities have ceased and the insertion are already stable [9]. The information on the insertion patterns will be very useful in determining the stability of the insertion and the type of insertion of each line for future insertion site identification.

### 3.2. Germination stage

Germination stage is started when the seeds are sown and lasting for 2 weeks after sowing. This stage is one of the most important stages in plant development [2]. Any stress or disruption in this stage could affect further development of the plant.

Among the 4 mutant lines, line 654 has the worst germination percentage, for both in saline and control non saline conditions. However, in the saline condition, 654 mutant lines germination was slightly better than its germination in non-saline condition (Figure 3A)

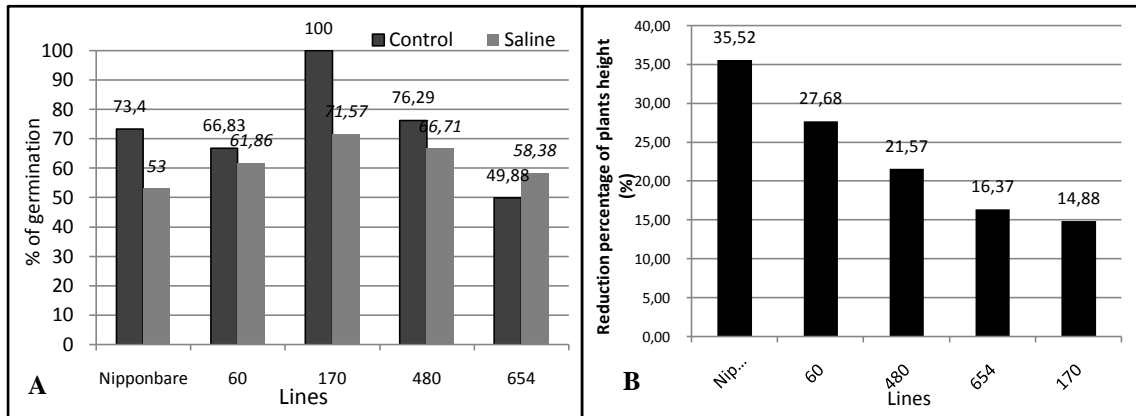


Fig 3. (A). Average of germination percentage of Nipponbare and 4 mutant lines two weeks-after-sowing. (*Control* = plants without saline treatment, *Saline* = plants with saline treatment.); (B). Reduction percentage of Nipponbare and 4 mutant lines plants height, two weeks-after-sowing.

The mutant line with the best performance during germination stage is line 170. This result showed that in general, all of rice mutant lines had better germination result in saline condition than the Nipponbare's as the wild type. Thus, all rice mutant line had lower germination reduction percentage than Nipponbare's. Rice mutant 170 has the highest germination percentage both in saline and non-saline condition (Figure 3A). It also has the lowest reduction rate (14.88%) for plant height (Figure 3B). The better plant condition during germination, the better development the plant will be. The vigour of the young plants can determine the strength of the plant in any stress condition. As seen in Figure 3B, Nipponbare as wildtype had the highest reduction percentage on plant height among the sample plants. This could be affected by the low germination condition of Nipponbare with saline media that only had shorts plant heights because of germination delay. On the contrary, rice mutant line 170 has the best germination percentage and growth both at saline and without saline media. Thus it has the lowest reduction percentage on plant height parameter. This condition could support the growth of the mutant line 170 against saline condition in the next stage(s) of development.

### 3.3. Vegetative stage

Vegetative stage of rice plants were started from germination to the heading time of flag leaf [2]. But in this experiment, to differentiate from the germinating stage, the vegetative stage was determined from the end of germination stage, at the 3-leave-stage. Plant heights and total number of tillers were measured at this stage.

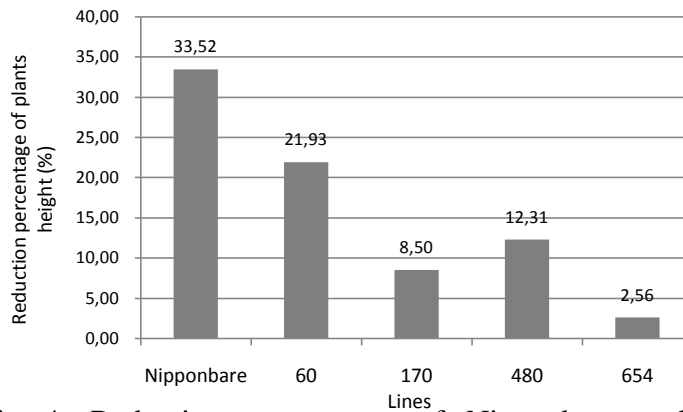


Fig 4. Reduction percentages of Nipponbare and 4 mutant lines heights after reproductive stage under salting treatments

The mutant line 654 has the lowest reduction percentage for plant height (2.56%). It means that the height of plant in saline condition is just slightly lower (2.56%) than the height of plant in non-saline condition (Fig 4). Even though 654 was in second place in plant height reduction percentage during germination; the plants in saline condition still could develop better than 170 in the next stage development. It shows that line 654 has specific mechanism to defense against the saline condition thus it can maintain the development of its height.

Tillering is another important development parameter. Under adverse condition, the number of rice tillers is more than the number of panicle [1]. It means that not all of the tillers is productive tillers. It could be there are more non-productive tillers than productive tillers in the stress condition. Lower percentage of productive tiller will affect the rice yield. In this experiment 60 mutant rice line has the best productivity percentage of tiller number when growing in saline condition (Table 2).

Table 2. Total productive tillers of Nipponbare and 4 mutant lines with and without saline treatments

Lines	Control			Saline treatment		
	Productive tillers	Total tillers	Productivity (%)	Productive tillers	Total tillers	Productivity (%)
NB	4.71	7.00	67.35	1.00	4.00	25.00
60	5.43	6.86	79.17	2.71	5.43	50.00
170	4.86	6.86	70.83	2.71	5.57	48.72
480	5.86	7.86	74.55	2.57	7.71	33.33
654	4.38	6.38	68.63	2.75	8.13	33.85

Control = plants without saline treatment, Saline = plants with saline treatment

### 3.4. Reproductive stage

Reproductive stage is started when the rice plant is in booting stage. But since it is not really visible, flag leaf heading was used as the indicator for the start of reproductive stage. For a crop, reproductive stage is important as it affects the yield of the crop [3].



Table 2. Flowering time of each Nipponbare and 4 mutant lines with and without saline treatments

Lines	Control	Saline treatment
NB	week 8-9	week 10-11
60	week 7	week 9
170	week 8-9	week 10-11
480	week 8-10	week 10-12
654	week 8-11	week 11-13

Control = plants without saline treatment, Saline = plants with saline treatment, week = week after sowing

Mutant line 60 has earlier flowering time than other mutant lines, and even earlier than Nipponbare, as wild type, in both conditions: saline and non-saline. Mutant line 170 has the same flowering to Nipponbare, while the flowering times of mutant line 480 and 654 are slower later than the wildtype, Nipponbare. Earlier flowering means shorter time of vegetative stage and earlier time to have yield. This trait is a favorable trait for a crop plant.

Table 3. Grain productivities of Nipponbare and 4 mutant lines growing in saline and non-saline condition

Lines	Grains						Reduction (%)
	Control			Saline Treatment			
	Total	Productive grains	Productivity (%)	Total	Productive grains	Productivity (%)	
NB	211.43	173.29	81.96	118.71	90.71	76.41	6.77
60	197.14	138.14	70.07	180.71	130.71	72.33	-3.22
170	207.57	153.86	74.12	157.57	124.00	78.69	-6.17
480	217.43	114.43	52.63	198.29	112.43	56.70	-7.74
654	226.14	159.63	70.59	201.43	262.36	130.25	-84.52

Control = plants without saline treatment, Saline = plants with saline treatment

Grain productivity is an important productive stage trait. It defines the amount of the yield of the crops. During stress condition, plants invest their energy for defense against the stress condition, thus the productivity usually is much lower than in normal condition (Ref). When a plant still has good productivity during any stress, the plant could be defined as tolerant to the stress. In this case, all of the 4 mutant lines showed better performance in grain productivity (Table 3) when grown in saline condition than in non-saline condition. It seems that all of the mutant lines need saline condition for better performance in grain productivity. This assumption needs to be proved. All of them have minus reduction percentage in the grain productivity. It means that the grain productivities of the mutant lines on saline treatment are better than the grain productivities of the lines in non-saline treatment. The best performance is the mutant line 654 with percentage of grain productivity at -84.52%.

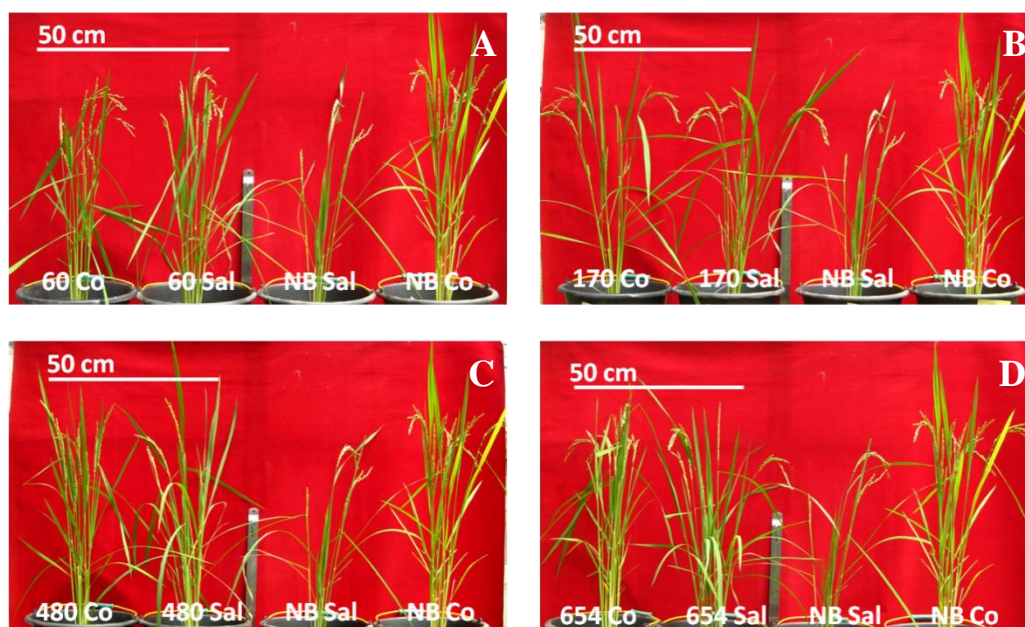


Figure 5. Performance of mutant lines and Nipponbare plants with and without saline treatments. **A.** 60 mutant line with the Nipponbare, **B.** 170 mutant line with the Nipponbare, **C.** 480 mutant line with the Nipponbare, **D.** 654 mutant line with the Nipponbare, Co = plants without saline condition (control), Sal = plants with saline condition.

#### 4. Conclusion

Each mutant line has specific superiority trait(s) to tolerate saline condition. It can be divided derived on rice life stages: germination, vegetative and production stage. The rice mutant line 170 is superior to other mutant lines on germination stage and has best productivity percentage of tiller number in saline condition. The rice mutant line 654 has the best plant height reduction and grain productivity in saline condition. The rice mutant line 60 is earlier to flower in saline and no saline condition than the Nipponbare and other mutant lines. It needs further salinity validation experiment to compare the saline tolerance of mutant lines to the saline tolerance of Pokkali (saline tolerant rice line).

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